

## Biosystematic Studies on Commelinaceae (Commelinales) I. Phylogenetic Analysis of *Commelina* in Eastern and Southeastern Asia

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*Commelina*, the pantropical and largest genus in Commelinaceae, consists of ca. 205 species with characteristic conduplicate involucre bracts. Previous phylogenetic studies of *Commelina*, which mainly used African and North American species, suggested that the ancestral character state of the margins of the involucre bracts of *Commelina* was free and that free to fused occurred only once. To test this evolutionary scenario, we performed parsimony and likelihood analyses with partial *matK* sequences using 25 individuals from 11 species of *Commelina*, primarily from eastern and southeastern Asia, with *Aneilema* and *Pollia* as outgroups. Results showed that *Commelina* comprises two major clades, one consisting of four species, and the other consisting of seven species. Species with free margins of the involucre bracts were in both major clades: *C. suffruticosa* in the first clade and *C. coelestis*, *C. communis*, *C. diffusa*, *C. purpurea* and *C. sikkimensis* in the latter. The phylogenetic trees suggested that the number of shifts is fewer when the ancestral state was fused and that there were two parallel evolutionary trends toward free.

Key words: *Commelina*, *Commelina maculata*, *Commelina paludosa*, *Commelina suffruticosa*, Commelinaceae, involucre bracts, *matK*, maximum likelihood, maximum parsimony, phylogeny

*Commelina* L., the largest genus of Commelinaceae Mirb. with ca. 205 species (Govaerts & Faden 2009), is widely distributed in tropical to temperate Africa, Asia, Australia, and North and South America (Faden 1998). Notable characteristics of *Commelina* are (1) folded spathe-like involucre bracts subtending two cincinni (Fig. 1B), (2) three upper staminodes with usually 4- or 6-lobed antherodes, (3) morphological differentiation between two lower lateral stamens and a lower central stamen, and (4) 2-3-locular and 2-3-valved capsules (Faden 1998).

Within Commelinaceae (41 genera, ca. 650 species), *Commelina* is classified in the tribe Commelineae Meisner (14 genera, ca. 350 species), which is composed of primarily old world genera (Faden 1998). A molecular phylogenetic analysis by Evans *et al.* (2003) showed *Commeli-*

*na* to be sister to a clade of *Pollia* Thunb., *Polyspatha* Benth., *Aneilema* R. Br. and *Rhopalephora* Hassk. These four genera share upper stamens that are more or less differentiated from the lower stamens, as in *Commelina*.

Eight species of *Commelina* (*C. communis* L., *C. diffusa* Burm. f., *C. suffruticosa* Blume, *C. auriculata* Blume, *C. benghalensis* L., *C. maculata* Edgew., *C. paludosa* Blume and *C. undulata* R. Br.) are known from eastern Asia (Hong & DeFilipps 2000). *Commelina sikkimensis* C. B. Clarke is in the northern part of Southeast Asia (Ridley 1924). These nine species are highly variable morphologically (Fig. 1). Based on our observations (Table 1), the margins of the involucre bracts are free in *C. communis*, *C. diffusa*, *C. suffruticosa* and *C. sikkimensis*, but fused into an infundibulum in the remaining five species (Fig. 1).

TABLE 1. Morphological characters and geographical distribution of the *Commelina* species analyzed in this study.

Species	Distribution	Margin of involucre bract	Number of seeds per ventral capsule locules	Color of petals	Shape of antherodes
<i>Commelina auriculata</i>	Asia	fused	1	blue	lobed
<i>C. benghalensis</i>	Asia and Africa	fused	2	blue	lobed
<i>C. coelestis</i> Willd.	North America	free	2	blue	lobed
<i>C. communis</i>	Asia	free	2	blue	lobed
<i>C. diffusa</i>	Pantropical	free	2	blue	lobed
<i>C. erecta</i>	North and South America	fused	1	blue	lobed
<i>C. maculata</i>	Asia	fused	1	blue	lobed
<i>C. paludosa</i>	Asia	fused	1	blue	lobed
<i>C. purpurea</i> C. B. Clarke	Africa	free	2	orange	lobed
<i>C. sikkimensis</i>	Asia	free	2	blue	lobed
<i>C. suffruticosa</i>	Asia	free	1	whitish	unlobed

*Commelina auriculata*, *C. benghalensis*, *C. maculata* and *C. paludosa* have one seed per locule (subgenus *Monoon* C. B. Clarke); the others have two seeds per locule (subgenus *Didymoon* C. B. Clarke). All have blue or lilac petals except *C. suffruticosa*, which has whitish petals (Fig. 1). Additionally, *C. suffruticosa* has such unusual features as vestigial unlobed antherodes (Fig. 1Ca) and a combination of involucre bracts with free margins and 1 seed per capsule locule.

The most recent phylogenetic analysis of *Commelina* (Burns *et al.* 2011) supported the monophyly of the genus, but showed neither sections *Monoon* nor *Didymoon* to be monophyletic. Instead, the species formed three major clades: (1) a clade with fused spathe (involucre bract) including all species analyzed of section *Monoon*, (2) a clade including the Asian species with the margins of the spathe free, and (3) a North American clade with the margins of the spathe free. The number of analyzed species distributed in Asia, however, was only three. The phylogenetic relationships among the Asian species remains unresolved. Burns *et al.* (2011) presumed that species of *Commelina* with fused spathes originated from species with free spathes only once, although the bootstrap values were too low to support their hypothesis.

The aims of this study were to reveal the phylogeny of *Commelina* in eastern and southeastern Asia and to reexamine the phylogenetic perspectives regarding the evolution of fused involucre

bracts as proposed by Burns *et al.* (2011).

## Materials and Methods

Twenty-two samples from eight species of *Commelina* (*C. auriculata*, *C. benghalensis*, *C. communis*, *C. diffusa*, *C. maculata*, *C. paludosa*, *C. sikkimensis* and *C. suffruticosa*) from northern southeastern and eastern Asia were analyzed (Table 2). The North American *C. coelestis* was also analyzed. Multiple samples were analyzed for a few species to determine the extent of intraspecific variation. We used *Polia japonica*, *P. thyrsoiflora*, and *Aneilema beniniense* as outgroups (for intergeneric relationships in Commelinaceae see Evans *et al.* 2003). For all of them, we determined 546 bp of DNA sequences of the plastid *matK* gene (partial). Two additional sequences of *Commelina* were obtained from GenBank (Table 2). We conducted parsimony and likelihood analyses and constructed molecular phylogenetic trees based on a matrix of 28 sequences.

Total genomic DNA was extracted from fresh or silica-gel-dried leaves. DNA extraction, PCR amplification, DNA sequencing and maximum parsimony (MP) analysis followed Tamura *et al.* (2010), and maximum likelihood (ML) analysis followed Tamura *et al.* (2011). All primers were newly designed: *matK746FCom* (5'-GAT AGA TCT TGG CAA CAG CA-3') and *matK2364RCom* (5'-AAT ATC CAA ATA CCA

TABLE 2. Sources of materials of *Commelina* and allied genera.

Species	Locality	Voucher	Acc. no. ( <i>matK</i> )
<i>Commelina auriculata</i>	Japan: Ishigaki Isl., Pref. Okinawa	<i>C.-K. Lee 85</i> (KYO)	LC323123
<i>C. benghalensis</i>	Japan: Mt. Nijo, Pref. Nara	<i>J. Oda 8900</i> (KYO)	LC323128
	Japan: Fukue Isl., Pref. Nagasaki	<i>Tamura &amp; Fuse 44008</i> (KYO)	LC323127
	Thailand: Doi Ang Khang, Prov. Chiang Mai	<i>Tamura et al. 30064</i> (KYO, BKF)	LC323129
<i>C. coelestis</i>	Unknown: cultivated in Kyoto Pref. Bot. Gard.	<i>C.-K. Lee 25</i> (KYO)	LC323130
<i>C. communis</i>	Japan: Kuchinoshima Isl., Pref. Kagoshima	<i>C.-K. Lee 11</i> (KYO)	LC323108
	Japan: Kiso-machi, Pref. Nagano	<i>C.-K. Lee 53</i> (KYO)	LC323124
	Japan: Shimokawa-mura, Pref. Kochi	<i>C.-K. Lee 94</i> (KYO)	LC323110
	Japan: Takahashi-shi, Pref. Okayama	<i>C.-K. Lee 98</i> (KYO)	LC323109
	Japan: Fukue Isl., Pref. Nagasaki	<i>Tamura &amp; Fuse 44005</i> (KYO)	LC323125
	Japan: Fukue Isl., Pref. Nagasaki	<i>Tamura &amp; Fuse 44006</i> (KYO)	LC323126
<i>C. diffusa</i>	Thailand: Khlong Lan National Park, Prov. Kamphaeng Phet	<i>Tamura et al. 20607</i> (KYO, BKF)	LC323116
	Thailand: Bangkok	<i>Tamura et al. 20630</i> (KYO, BKF)	LC323117
<i>C. erecta</i> *	USA: Florida	<i>J. R. Abbot 22649</i> (FLAS)	KJ772671
<i>C. maculata</i>	Thailand: Doi Chiang Dao, Prov. Chiang Mai	<i>Tamura et al. 30918</i> (KYO)	LC323113
	Thailand: Doi Chiang Dao, Prov. Chiang Mai	<i>Tamura et al. 30966</i> (KYO, BKF)	LC323114
<i>C. paludosa</i>	Thailand: Khlong Lan National Park, Prov. Kamphaeng Phet	<i>Tamura et al. 20601</i> (KYO, BKF)	LC323111
	Thailand: Khun Pa Wor National Park, Prov. Tak	<i>Tamura et al. 20621</i> (KYO, BKF)	LC323112
<i>C. purpurea</i> *	Unknown	<i>NMNH 94-904</i> (US)	GQ248103
<i>C. sikkimensis</i>	Thailand: Khlong Lan National Park, Prov. Kamphaeng Phet	<i>Tamura et al. 20603</i> (KYO, BKF)	LC323115
	Thailand: Doi Ang Khang, Prov. Chiang Mai	<i>Tamura et al. 30069</i> (KYO, BKF)	LC323118
	Thailand: Doi Chiang Dao, Prov. Chiang Mai	<i>Tamura et al. 30932</i> (KYO)	LC323119
	Thailand: Doi Chiang Dao, Prov. Chiang Mai	<i>Tamura et al. 30969</i> (KYO, BKF)	LC323120
<i>C. suffruticosa</i>	Thailand: Mae Wong National Park, Prov. Nakhon Sawan	<i>Tamura et al. 20609</i> (KYO, BKF)	LC323121
	Thailand: Doi Ngu, Prov. Chiang Rai	<i>Tamura et al. 30050</i> (KYO)	LC323122
<i>Aneilema acuminatum</i> R. Br.	Unknown: cultivated in Kyoto Pref. Bot. Gard.	<i>C.-K. Lee 20</i> (KYO)	LC323131
<i>Polia japonica</i> Thunb.	Japan: Sakyo-ku, Pref. Kyoto	<i>C.-K. Lee 84</i> (KYO)	LC323133
<i>P. thyrsiflora</i> (Blume) Steud.	Thailand: Khun Pa Wor National Park, Prov. Tak	<i>Tamura et al. 20617</i> (KYO, BKF)	LC323132

\* indicates taxa for which sequence was obtained from Genbank.

AAT GCG-3') for PCR amplification; matK-1339FCom (5'-ACG AAT ATC TTA ATT ATT GGA AAA GT-3'), matK1739RCom (5'-TCC TTG ATA TCG AAC ATA ATG-3') and matK2364RCom for DNA sequencing. For MP analysis, we used PAUP\* v. 4.0 beta 10 (Swofford 2002) to find the most parsimonious tree (heuris-

tic; 10,000 random) and also to perform bootstrap analysis with 10,000 replications (heuristic; 100 random). For ML analysis, the models selected by the hierarchical likelihood ratio test (hLRT) and Akaike information criterion (AIC) in Modeltest v. 3.7 (Posada & Crandall 1998) were identical: TVM. The model was implemented in PAUP\*

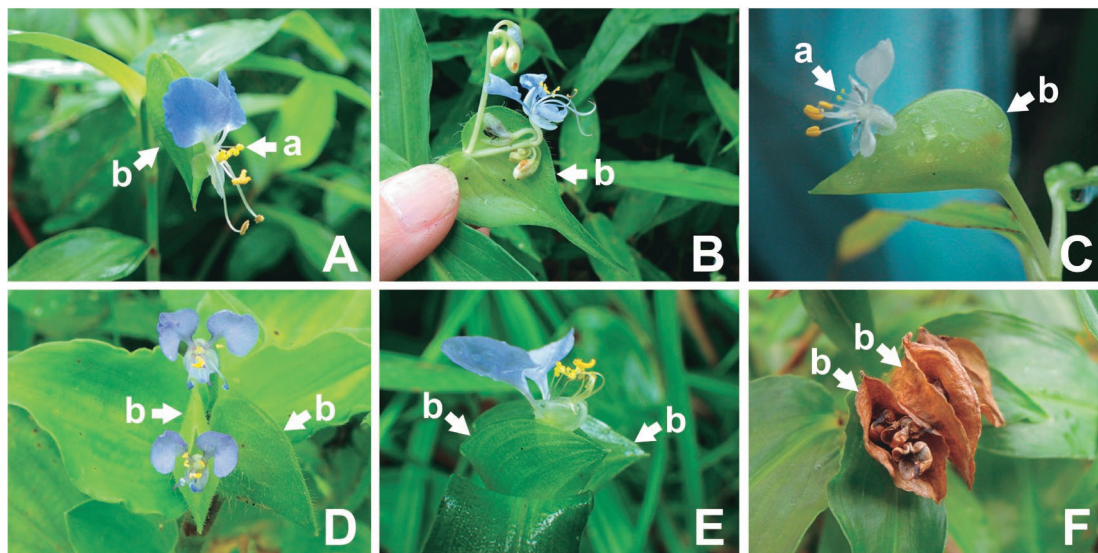


FIG. 1. Reproductive organs and involucral bracts of *Commelina* species in eastern and southeastern Asia. A, *C. communis*; B, *C. sikkimensis* with an opened involucral bract to show the two cincinni; C, *C. suffruticosa*; D, *C. benghalensis*; E, *C. maculata*; F, *C. maculata* with mature capsules and brown involucral bracts. a, antherode; b, involucral bract.

(heuristic; 10,000 random). ML bootstrap analysis with 10,000 replication (heuristic; 100 random) was performed also using PAUP\*. Identification of the species followed Hong & DeFilipps (2000) and Noltie (1994). Voucher specimens were deposited in the herbaria KYO and BKF.

## Results

Of the 546 aligned bp of *matK*, 61 bp were variable, and 37 bp were phylogenetically informative. The MP analysis of the *matK* sequences yielded a single most parsimonious tree of 65 steps. The consistency index (CI), retention index (RI) and rescaled consistency index (RC) values for each tree were 0.95, 0.97 and 0.92, respectively.

The topology of the ML tree was identical with that of the MP tree. The monophyly of the genus *Commelina* was strongly supported (100 % BS in MP and ML). According to the MP strict consensus tree as well as the ML trees (Fig. 2), *Commelina* consists of two major clades; the first major clade received 97% (MP) and 95% (ML) bootstrap support (BS). The second major clade received 77–81% BS. In the first major clade, one

lineage comprising *C. paludosa* and *C. maculata* diverged as the first branch, followed successively by *C. coelestis*, *C. communis*, and a lineage of *C. diffusa*, *C. purpurea* and *C. sikkimensis*. In the second major clade, *C. benghalensis* was sister to the remainder, in which *C. auriculata* was sister to a clade of *C. erecta* and *C. suffruticosa*.

## Discussion

### Phylogenetic relationships

The phylogenetic trees generated in this study were not incongruent with those of Burns *et al.* (2011): three lineages presented in Burns *et al.* (2011), including (1) *Commelina coelestis*, (2) *C. erecta* — *C. benghalensis*, and (3) *C. communis* — *C. diffusa* — *C. purpurea*, were confirmed in our phylogenetic analysis. Further, we revealed that lineages 1 and 3 are related (BS = 86–87%). In Burns *et al.* (2011), the Asian species were only in lineage 3, except for *C. benghalensis* with an African-Asian distribution. However, we revealed that lineage 2 also includes Asian species and that the Asian species *C. paludosa* and *C. maculata* were absent in the three lineages.

Previous studies based on karyology and

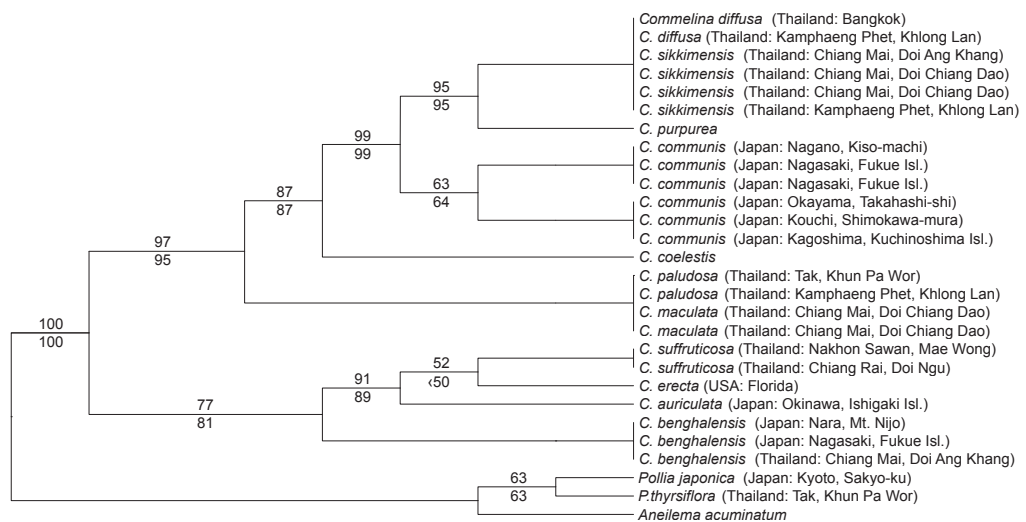


FIG. 2. A single most parsimonious tree resulting from the maximum parsimony (MP) analysis of plastid *matK* (partial) DNA sequence data of *Commelina* and outgroup genera. The topology of the maximum likelihood (ML) tree is identical with the topology of this figure. Numbers above branches indicate MP bootstrap values. Numbers below branches indicate ML bootstrap values.

morphology revealed two strains of *Commelina communis* (Fukumoto 1965, Fujishima 2003), one representing a strain of tetraploids with hairy involucre bracts, and the other representing a strain of octoploids with hairless involucre bracts. Our *matK* sequence data (partial) were also variable within *C. communis*. Further studies are needed to clarify whether the two strains of *C. communis* represent distinct taxa. In contrast with *C. communis*, there were no differences in the *matK* (partial) sequences of *C. diffusa* and *C. sikkimensis*. *Commelina sikkimensis* was recently proposed as a variety of *C. diffusa* because they are discriminated only by the shape of the involucre bract and seed surface sculpturing (Nandikar 2013). Future molecular studies may help to determine whether they are conspecific.

*Commelina auriculata* and *C. suffruticosa* were more closely related to the American *C. erecta* than to the Afro-Asian *C. benghalensis*. *Commelina auriculata* and *C. erecta* share auriculate leaf sheaths and capsule locules with one seed with the Asian-Australian *C. undulata* (not analyzed in this study), which is very similar to *C. erecta* morphologically. The close phylogenetic relationship of *C. suffruticosa* with *C. auricu-*

*lata* and *C. erecta* was unexpected. Despite sharing subglobose capsules with 1-seeded locules, *C. suffruticosa* differs morphologically from *C. auriculata* and *C. erecta* in having several autapomorphies, such as involucre bracts with free margins and leaf sheaths without auricles. *Commelina suffruticosa* has small whitish petals with highly reduced antherodes, which are unusual features in *Commelina*, making it easily distinguishable from other species. Considering that *Commelina* attracts pollinators visually by colored petals and that the conspicuous antherodes function as resources of rewards (Faden 1992, Ushimaru *et al.* 2007), such floral modifications of *C. suffruticosa* appear to be related to less frequent outcrossing.

#### Evolution of involucre bracts

Burns *et al.* (2011) hypothesized, mainly by analyzing African species, that fused involucre bracts evolved from bracts with free margins only once in *Commelina*. Our analysis of the Asian species, however, indicates that hypothesis may not be correct. One of the two aforementioned major clades, except for *C. suffruticosa* which has involucre bracts with free margins, is

occupied by species with marginally fused funnel-shaped involucre bracts. *Commelina suffruticosa* is embedded deep in this clade, and is sister to *C. erecta*, whose involucre bracts are fused. Another major clade has two lineages: one consisting of *C. maculata* and *C. paludosa*, whose involucre bracts are fused, and the other consisting of *C. coelestis*, *C. communis*, *C. purpurea*, *C. sikkimensis*, and *C. diffusa*, whose involucre bracts are free. The character-state distribution suggests that the evolution of involucre bracts is not as simple as presented by Burns *et al.* (2011) and cannot be explained by only a single shift from free to fused bracts. We revealed that the fewest number of shifts within the genus was two, requiring fused margins of the involucre bracts as the ancestral state, followed by two independent evolutionary pathways from fused to free: once in *C. suffruticosa* and again in the clade of *C. coelestis*, *C. communis*, *C. diffusa*, *C. purpurea* and *C. sikkimensis*. If the ancestral state of the margins of the involucre bracts of *Commelina* is free, as assumed by Burns *et al.* (2011), one additional step is required. Further molecular phylogenetic studies using more extensive samples and also comparative morphological studies of the involucre bracts of *Commelina* and related groups are needed for understanding trends in involucre bract evolution. The ecological significance of fusion of the involucre bracts is also unclear and needs further investigation.

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